Patent claims

1. Process for the preparation of L-threonine using bacteria of the Enterobacteriaceae family which produce L-threonine, characterized in that

   a) the bacterium is inoculated and cultured in at least a first nutrient medium,

   b) at least a further nutrient medium or further nutrient media is/are then fed continuously to the culture in one or several feed streams, the further nutrient medium or the further nutrient media comprising at least one source of carbon, at least one source of nitrogen and at least one source of phosphorus, under conditions which allow the formation of L-threonine, and at the same time culture broth is removed from the culture with at least one or several removal streams which substantially corresponds/correspond to the feed stream or the total of the feed streams, wherein

   c) the concentration of the source of carbon during the continuous culturing in step b) is adjusted to not more than 30 g/l.

2. Process according to claim 1, wherein the culturing step (a) is carried out by the batch process.

3. Process according to claim 1, wherein the culturing step (a) is carried out by the fed batch process, at least one additional nutrient medium being employed.

4. Process according to claim 1, 2 or 3, wherein the L-threonine formed is purified.

5. Process according to claim 1, 2 or 3, wherein in step (b) the biomass is first removed to the extent of at

least 90% from the culture removed, and the water is then removed to the extent of at least 90%.

6. Process according to claim 1 or 2, wherein the further nutrient medium or the further nutrient media is (are) fed in after >0 to 20 hours, with respect to the start of the batch process.

7. Process according to claim 1 or 3, wherein the further nutrient medium or the further nutrient media is (are) fed in after >0 to 80 hours, with respect to the start of the fed batch process.

8. Process according to claim 1, wherein the source of carbon is one or more of the compounds chosen from the group consisting of sucrose, molasses from sugar beet or cane sugar, fructose, glucose, starch hydrolysate, cellulose hydrolysate, arabinose, maltose, xylose, acetic acid, ethanol and methanol.

9. Process according to claim 1, wherein the source of nitrogen is one or more organic nitrogen-containing substances or substance mixtures chosen from the group consisting of peptones, yeast extracts, meat extracts, malt extracts, corn steep liquor, soya bean flour and urea and/or one or more of the inorganic compounds chosen from the group consisting of ammonia, ammonium-containing salts and salts of nitric acid.

10. Process according to claim 9, wherein the ammonium-containing salts and salts of nitric acid are ammonium sulfate, ammonium chloride, ammonium phosphate, ammonium carbonate, ammonium nitrate, potassium nitrate and potassium sodium nitrate.

11. Process according to claim 1, wherein the source of phosphorus is phosphoric acid or polymers thereof or phytic acid or alkali metal or alkaline earth metal salts thereof.

12. Process according to claim 11, wherein the alkali metal salts of phosphoric acid are potassium dihydrogen phosphate or dipotassium hydrogen phosphate or the corresponding sodium-containing salts.

13. Process according to claim 1, wherein the speed of the removal stream or the removal streams corresponds to 80% - 120%, 90% - 110% of the feed stream or of the total of the feed streams.

14. Process according to claim 1, wherein the start of the removal or the removals takes place at the same time as or with a time shift relative to the feed or the total of the feeds.

15. Process according to claim 1, wherein the bacteria of the Enterobacteriaceae family are the species Escherichia coli.

16. Process according to claim 1, wherein the bacterium of the Enterobacteriaceae family contains at least one thrA gene or allele which codes for a threonine-insensitive aspartate kinase I - homoserine dehydrogenase I.

17. Process according to claim 1, wherein the bacterium of the Enterobacteriaceae family contains a stop codon chosen from the group consisting of opal, ochre and amber, preferably amber, in the rpoS gene and a t-RNA suppressor chosen from the group consisting of opal suppressor, ochre suppressor and amber suppressor, preferably amber suppressor.

18. Process according to claim 1, wherein the feed stream or the total of the feed streams is fed in at a rate corresponding to an average residence time of less than 30 hours, less than 25, less than 20 hours.

19. Process according to claim 1, wherein in the nutrient medium fed in or the nutrient media fed in a phosphorus to carbon ratio (P/C ratio) of not more than 4; of not more than 3; of not more than 2; of not more than 1.5; of not more than 1; of not more than 0.7; of not more than 0.5; of not more than 0.48; of not more than 0.46; of not more than 0.44; of not more than 0.42; of not more than 0.40; of not more than 0.38; of not more than 0.36; of not more than 0.34; of not more than 0.32; or of not more than 0.30 is established.

20. Process according to claim 1, wherein the culture broth removed is provided with oxygen or an oxygen-containing gas until the concentration of the source of carbon falls below 2 g/l; below 1 g/l; below 0.5 g/l.

21. Process according to claim 17, wherein the L-threonine formed is purified.

22. Process according to claim 17, wherein in step (b) the biomass is first removed to the extent of at least 90% from the culture removed, and the water is then removed to the extent of at least 90%.

23. Process according to claim 1, 2 or 3, wherein the concentration of the source of carbon during the culture is adjusted to not more than 20, 10 or 5 g/l.

24. Process according to claim 1, 2 or 3, wherein the concentration of the source of carbon during the culture is adjusted to not more than 5 or 2 g/l.

25. Process according to claim 23 or 24, wherein the concentration of the source of carbon during the culture is adjusted to not more than 5 g/l.

26. Process according to claim 23 or 24, wherein the concentration of the source of carbon during the culture is adjusted to not more than 2 g/l.

27. Process according to claim 1, 2 or 3, wherein the yield of L-threonine formed, based on the source of carbon employed, is at least 31%.

28. Process according to claim 1, 2 or 3, wherein the yield of L-threonine formed, based on the source of carbon employed, is at least 37 %.

29. Process according to claim 1, 2 or 3, wherein the yield of L-threonine formed, based on the source of carbon employed, is at least 42 %.

30. Process according to claim 1, 2 or 3, wherein L-threonine is formed with a space/time yield of at least 1.5 to 2.5 g/l per h.

31. Process according to claim 1, 2 or 3, wherein L-threonine is formed with a space/time yield of at least 2.5 to more than 3.5 g/l per h.

32. Process according to claim 1, 2 or 3, wherein L-threonine is formed with a space/time yield of at least 3.5 to 5.0 g/l per h.

33. Process according to claim 1, wherein a fed batch process is used in the culturing step (a), and in that L-threonine is formed with a space/time yield of at least 5.0 to more than 8.0 g/l per h.

34. Sucrose-utilizing transconjugants of Escherichia coli K-12 deposited as DSM 16293 at the Deutsche Sammlung für Mikroorganismen und Zellkulturen [German Collection of Microorganisms and Cell Cultures] (Braunschweig, Germany).

35. Process according to claim 1, 2 or 3, wherein strains which have at least the following features are employed:

a) a threonine-insensitive aspartate kinase I - homoserine dehydrogenase I, which is optionally present in overexpressed form, and

b) a stop codon chosen from the group consisting of opal, ochre and amber, preferably amber in the rpoS gene, and a t-RNA suppressor chosen from the group consisting of opal suppressor, ochre suppressor and amber suppressor.

36. Process according to claim 1, 2 or 3, wherein strains which have at least the following features are employed:

a) a threonine-insensitive aspartate kinase I - homoserine dehydrogenase I, which is optionally present in overexpressed form,

b) are not capable, under aerobic culture conditions, of breaking down threonine,

c) an at least partial need for isoleucine, and

d) growth in the presence of at least 5 g/l threonine.

37. Process according to claim 1, 2 or 3, wherein strains which have at least the following features are employed:

a) a threonine-insensitive aspartate kinase I - homoserine dehydrogenase I, which is optionally present in overexpressed form,

b) a stop codon chosen from the group consisting of opal, ochre and amber, preferably amber in the

rpoS gene, and a t-RNA suppressor chosen from the group consisting of opal suppressor, ochre suppressor and amber suppressor,

c) are not capable, under aerobic culture conditions, of breaking down threonine, preferably by attenuation of threonine dehydrogenase,

d) an at least partial need for isoleucine, and

e) growth in the presence of at least 5 g/l threonine.

38. Process according to claim 35, 36 or 37, wherein the strain employed additionally contains one or more of the features chosen from the group consisting of

38.1 attenuation of phosphoenol pyruvate carboxykinase, which is coded by the pckA gene,

38.2 attenuation of phosphoglucose isomerase, which is coded by the pgi gene,

38.3 attenuation of the YtfP gene product, which is coded by the open reading frame ytfP,

38.4 attenuation of the YjfA gene product, which is coded by the open reading frame yjfA,

38.5 attenuation of pyruvate oxidase, which is coded by the poxB gene,

38.6 attenuation of the YjgF gene product, which is coded by the open reading frame yjgF,

38.7 enhancement of transhydrogenase, which is coded by the genes pntA and pntB,

38.8 enhancement of phosphoenol pyruvate synthase, which is coded by the pps gene,

38.9 enhancement of phosphoenol pyruvate carboxylase, which is coded by the ppc gene.

38.10 enhancement of the regulator RseB, which is coded by the rseB gene,

38.11 enhancement of the galactose proton symporter, which is coded by the galP gene,

38.12 ability to be able to use sucrose as a source of carbon,

38.13 enhancement of the YedA gene product, which is coded by the open reading frame yedA,

38.14 growth in the presence of at least 0.1 to 0.5 mM or at least 0.5 to 1 mM borrelidin (borrelidin resistance),

38.15 growth in the presence of at least 2 to 2.5 g/l or at least 2.5 to 3 g/l diaminosuccinic acid (diaminosuccinic acid resistance),

38.16 growth in the presence of at least 30 to 40 mM or at least 40 to 50 mM α-methylserine (α-methylserine resistance),

38.17 growth in the presence of not more than 30 mM or not more than 40 mM or not more than 50 mM fluoropyruvic acid (fluoropyruvic acid sensitivity),

38.18 growth in the presence of at least 210 mM or at least 240 mM or at least 270 mM or at least 300 mM L-glutamic acid (glutamic acid resistance),

38.19 an at least partial need for methionine,

38.20 an at least partial need for m-diaminopimelic acid,

38.21 growth in the presence of at least 100 mg/l rifampicin (rifampicin resistance),

38.22 growth in the presence of at least 15 g/l L-lysine (lysine resistance),

38.23 growth in the presence of at least 15 g/l methionine (methionine resistance),

38.24 growth in the presence of at least 15 g/l L-aspartic acid (aspartic acid resistance), and

38.25 enhancement of pyruvate carboxylase, which is coded by the pyc gene